FINAL TECHNICAL REPORT
NASA GRANT # NAG 2-391
Covering the Period May 1989 - January 1993

Continuing Studies on Fluid Movement in Bone: Its Relationship to Mineral Dynamics and Spaceflight Osteopenia

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From research funded by previous NASA grants we have accrued a great deal of evidence in support of our original hypothesis regarding the effect of tail suspension in the rat on blood flow to the bones of the hindlimbs. Moreover, we have accumulated circumstantial evidence that these changes are causally related to the changes in bone mass associated with suspension in juvenile rats.

We employed the rat tail suspension model for simulating the effects of microgravity. We used littermate, juvenile rats for our studies in order to reduce individual variability and to take advantage of the higher rates of deposition and resorption of bone in the young animals. Our data concurred with earlier studies in showing a decrease in the tibial and femoral weights in suspended relative to control rats, with little or no change in the weights of the humerus or radius and ulna. We further demonstrated, however, that suspended animals had an increased skull and mandible mass. These data suggested that the altered distribution of blood flow was at least partially responsible for the redistribution of bone mass in the suspended animals (Roer and Dillaman, 1990).

We used finite difference, computer modeling of ISF flow in the matrix of bone using newly accrued morphological data. The new model employed plenum flow between the endosteal and periosteal surfaces of the bone, with flow being governed, in large part, by the difference in medullary and external ISF pressures, the mean porosity of the matrix and canaliculi, and the architecture of the vascular elements (Dillaman et al., 1991). Incidentally, the estimates of porosity which we used were recently substantiated by empirical observations and finite element modeling by Beaudoin et al. (1991). The model predicted that alterations in the blood supply to the endosteal surface of the bone should have important effects on the perfusion of osteocytes within the mineralized matrix. We have also begun a series of collaborations with Dr. Russell Keanini of the University of North Carolina at Charlotte to employ finite element modeling of bone fluid flow. Such analysis is capable of predicting the same flow patterns as that observed empirically by marker injection (Keanini et al., in submission to J. Fluid Mech.). We are thus able to predict the effects of reduced blood flow to bone on bone perfusion.

These predictions were further explored empirically by observing the rate of appearance and washout of intravenously injected horseradish peroxidase (HRP) in the femora of tail-suspended and control rats. At 5 min. after injection, HRP was evident along the endosteal margins of the femur in control rats, but not in the bones of the suspended animals. The difference was even more marked at 15 min. postinjection. Washout of HRP from the bones of suspended animals was also slower; little HRP evident in control femurs by 4 h. postinjection, while suspended rats still displayed significant amounts of HRP in the endosteal bone. The data, although qualitative, demonstrated that the rate at which HRP entered and washed out of the matrix of suspended rats was substantially slower than that of controls. These data suggest that the matrix of suspended rats experiences a reduced perfusion by ISF (Dillaman et al., 1991).

In order to link the putative reduction in matrix perfusion with a reduced blood flow to the bone, as predicted by the computer model, we have employed an ultrasonic, transit-time flow

probe and meter to measure femoral artery blood flow. Data have shown a marked reduction (on the order of 40%) in the rate of blood flow through the femoral artery of rats immediately upon suspension (Fig. 1). The effect persisted for up to a week of suspension, and was the same regardless of whether or not the rats had been suspended prior to the implantation of the probe. The reduction in flow was fully reversible upon return to normal posture (Fig. 1).

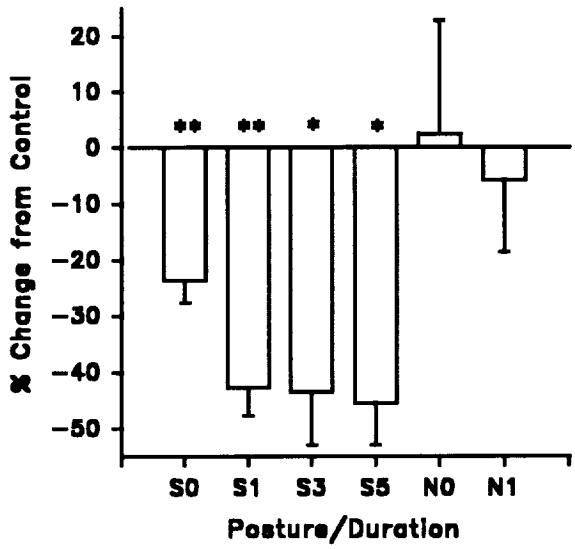

Fig. 1 Femoral artery flow on suspension (S0); 1, 3 & 5 d suspension (S1, S3, S5), return to normal (N0), 1 d normal posture (N1). Bars=SEM, *P<0.05, **P<0.005

To determine if growing rats are capable of compensating for the effects of suspension on blood flow by altering the pattern and/or density of bone blood vessels, we undertook a study of the vascular architecture within the femora of suspended and control juvenile rats. While at present limited to the mid-diaphysis of the femur, extensive sampling of serial sections and 3-dimensional reconstruction of those profiles enabled us to describe, both qualitatively and quantitatively, any morphological changes associated with 3-wk. suspension. This region was used, in part, because there were clearly identifiable landmarks that could insure that the same region was being examined from animal to animal. One hundred serial sections were obtained from each block to be used in the comparison of vascular canal volumes between suspended and control animals. The effects of suspension on bone morphological parameters were analyzed using two computer analysis programs, Image 1 (Universal Imaging Corp., Media, PA) and PC3D (Multidimensional Computing Inc., Durham, NC). Both programs converted images from the stained sections into polygons that could be measured by a personal computer. Of the 100 serial sections, every fourth section was used for analysis. The data suggest that there is no alteration of vascular density or dimensions of vessels, but a reduction in bone thickness. It would appear, therefore, that a constant vascular volume is maintained in cortical bone, regardless of the blood supply. The tendency for the numbers of vascular canals per unit area to remain relatively constant despite a 25% decrease in cortical thickness, seems to suggest that a basic unit is being used to construct the cortical bone. In this case the animal is responding to the decreased perfusion seen in suspension to make fewer units and therefore, less bone.

In order to assess the pattern and rate of bone deposition during recovery from a 3-wk. suspension in juvenile rats, we have used a battery of fluorescent bone-seeking markers (e.g. tetracycline, calcein, etc.) to record incremental bone growth. Animals were injected at weekly intervals for 3 weeks, beginning at the end of suspension, and were sacrificed for bone analysis after that period. We are currently analyzing these data using image analysis and stereological

techniques.

We have also developed an organ perfusion system that has allowed us to monitor the growth, calcium turnover, and metabolic activity of chick calvariae over time. This system has allowed us to begin a controlled investigation of the various parameters related to flow that can mediate flow effects on bone. The effect of living cells, pH and decreased perfusion on ^{45}Ca release was investigated in static cultures and the isolated bone perfusion system. Calvariae with living cells released more ^{45}Ca than devitalized calvariae. Release of ^{45}Ca from chick calvariae perfused at 15μl min^{-1} was inversely proportional to pH in the range of 7.4 to 6.7. Calcium release in response to decreased pH was also evident in static cultures, although less than perfused cultures. A decrease in the perfusion rate from 15μl min^{-1} to 2.5μl min^{-1} resulted in a drop in pH of the effluent medium of calvariae cultured in unbuffered medium, but not buffered medium. Release of ^{45}Ca at the lower perfusion rate, however, was greater in calvariae perfused with either buffered or unbuffered medium. The observation of an increase in ^{45}Ca release with decreased bone perfusion suggests that the production of protons by bone cells may represent a major link between decreased bone perfusion and osteopenia.

In summary, we have demonstrated that: 1) tail-suspension results in a 40% reduction in femoral artery blood flow; 2) computer modeling and extravascular marker data reveal that this reduction in blood flow results in a reduced perfusion of bone; 3) reduction in bone perfusion, *in vitro*, leads to a reduction in bone pH with a consequent release of bone calcium; 4) the response of rat bone to such the reduced blood flow and perfusion is to reduce cortical thickness by approximately 25%, without altering vascular density or architecture. These results firmly establish blood flow and bone perfusion as important factors in the etiology of spaceflight osteopenia.

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